CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising:

a translation system;

an orthogonal aminoacyl-tRNA synthetase (O-RS) selected from the group consisting of: an orthogonal tryptophanyl-tRNA synthetase (O-TrpRS), an orthogonal mutant tryptophanyl-tRNA synthetase (O-muTrpRS), and a derivative thereof; and,

an orthogonal tRNA (O-tRNA);

wherein the O-RS preferentially aminoacylates the O-tRNA with an amino acid or unnatural amino acid.

2. The composition of claim **1**, wherein the translation system comprises a cell or an *in vitro* translation system.

3. The composition of claim **2**, wherein the cell comprises a eukaryotic cell, a *Xenopus* cell, or a mammalian cell.

4. The composition of claim **2**, wherein the ***in vitro*** translation comprises a cell lysate.

5. The composition of claim **1**, wherein the O-RS is encoded by a nucleic acid comprising a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 1, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

6. The composition of claim **1**, wherein the O-RS comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 2, and a conservative substitution thereof.

7. The composition of claim **1**, wherein the unnatural amino acid comprises: a tryptophan analog or 5-hydroxy-L-tryptophan (5-HTPP).

8. The composition of claim **1**, wherein the O-RS comprises one or more improved or enhanced enzymatic properties, selected from the group consisting of: Km and Kcat, for the unnatural amino acid as compared to a natural amino acid.

9. The composition of claim **1**, wherein the O-tRNA is not substantially aminoacylated by an endogenous aminoacyl-tRNA synthetase of the translation system.

10. The composition of claim **1**, wherein the O-tRNA comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

11. The composition of claim **1**, wherein the O-tRNA recognizes a selector codon.

12. The composition of claim **11**, wherein the selector codon comprises a sequence selected from the group consisting of: a four base codon, a rare codon, UAG, UAA, and UGA.

13. The composition of claim **1**, further comprising a nucleic acid encoding a product peptide.

14. The composition of claim **13**, wherein the nucleic acid comprises a selector codon sequence recognized by the O-tRNA.

15. The composition of claim **13**, wherein the product peptide comprises an amino acid sequence that is at least 75% identical to that of a wild type therapeutic protein, a diagnostic protein, an industrial enzyme, or a portion thereof.

16. A composition comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates a tRNA with 5-hydroxy-L-tryptophan (5-HTPP).

17. The composition of claim **16**, wherein the O-RS is encoded by a nucleic acid comprising a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 1, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

18. The composition of claim **16**, wherein the O-RS comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 2, and a conservative substitution thereof.

19. The composition of claim **16**, wherein the O-RS comprises one or more improved or enhanced enzymatic properties, selected from the group consisting of: Km and Kcat, for aminoacylation with the 5-HTPP as compared to a tryptophan.

20. The composition of claim **16**, wherein the t-RNA is an O-tRNA.

21. The composition of claim **20**, wherein the O-tRNA is not substantially aminoacylated by an endogenous aminoacyl-tRNA synthetase.

22. The composition of claim **20**, wherein the O-tRNA comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

23. The composition of claim **20**, wherein the O-tRNA recognizes a selector codon.

24. The composition of claim **23**, wherein the selector codon comprises a sequence selected from the group consisting of: a four base codon, a rare codon, UAG, UGA, and UAA.

25. The composition of claim **16**, further comprising an endogenous translation system.

26. The composition of claim **25**, wherein the endogenous translation system comprises a cell or an *in vitro* translation system.

27. The composition of claim **26**, wherein the cell comprises eukaryotic cells or mammalian cells.

28. The composition of claim **16**, further comprising a nucleic acid encoding a product peptide.

29. The composition of claim **28**, wherein the t-RNA is an O-tRNA, and the nucleic acid comprises a selector codon sequence recognized by the O-tRNA.

30. The composition of claim **28**, wherein the product peptide comprises an amino acid sequence that is at least 75% identical to that of a wild type therapeutic protein, a diagnostic protein, an industrial enzyme, or a portion thereof.

31. A polypeptide comprising an amino acid sequence encoded by a coding polynucleotide sequence, the coding polynucleotide sequence selected from the group consisting of:

a) a coding polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, and a conservative variation thereof;

b) a coding polynucleotide sequence that encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, and a conservative substitution thereof;

c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially an entire length of a polynucleotide sequence of (a) or (b);

d) a complementary sequence of (a), (b), or (c); and,

wherein the polypeptide comprises an aminoacyl-tRNA synthetase activity charging with 5-HTPP.

32. A nucleic acid comprising: a polynucleotide sequence selected from the group consisting of:

a) a polynucleotide sequence selected from SEQ ID NO: 3, or a complementary polynucleotide sequence thereof;

b) a conservative variation of (a) that recognizes a selector codon; and,

c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a), and which comprises a tRNA that recognizes a selector codon.

33. The nucleic acid of claim **32**, wherein the selector codon is selected from the group consisting of: a four base codon, a rare codon, UGA, UAA, and UAG.

34. A method of incorporating an amino acid or unnatural amino acid into a peptide, the method comprising:

preparing a construct comprising a nucleic acid sequence encoding an orthogonal mutant tryptophanyl-tRNA synthetase (O-muTrpRS) or a derivative thereof;

preparing a construct comprising a nucleic acid sequence encoding an orthogonal tRNA (O-tRNA);

introducing the O-muTrpRS construct and the O-tRNA construct into a eukaryotic cell; and,

preferentially aminoacylating an expressed O-tRNA with the amino acid or unnatural amino acid, wherein said aminoacylation is catalyzed by an expressed O-muTrpRS;

whereby the amino acid or unnatural amino acid is incorporated into the peptide in the cell.

35. The method of claim **34**, wherein the unnatural amino acid comprises a tryptophan analog or 5-hydroxy-L-tryptophan (5-HTPP).

36. The method of claim **35**, further comprising applying a voltage to the peptide, thereby reacting the 5-HTPP with a reactive molecule.

37. The method of claim **36**, wherein reacting comprises cross-linking.

38. The method of claim **36**, wherein the reactive molecule comprises an unnatural amino acid in another peptide.

39. The method of claim **34**, further comprising detecting an interaction between the peptide and another peptide.

40. The method of claim **39**, wherein said detecting comprises fluoroscopy.

41. The method of claim **34**, wherein the O-muTrpRS construct comprises a nucleic acid comprising a polynucleotide sequence selected from the group consisting of:

a) a coding polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, and a conservative variation thereof;

b) a coding polynucleotide sequence that encodes a polypeptide selected from the group consisting of SEQ ID NO: 2, and a conservative substitution thereof;

c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially an entire length of a polynucleotide sequence of (a) or (b);

d) a complementary sequence of (a), (b), or (c); and,

e) pVal144ProBsTrpRS.

42. The method of claim **34**, wherein the O-muTrpRS construct comprises a mutated tryptophanyl-tRNA synthetase peptide sequence mutated at one or more amino acid residues based on structure data of the tryptophanyl-tRNA synthetase or an analogous aminoacyl-tRNA synthetase.

43. The method of claim **42**, wherein the mutated tryptophanyl-tRNA synthetase comprises a *Bacillus* tryptophanyl-tRNA synthetase mutated at Val144.

44. The method of claim **34**, wherein the O-tRNA construct comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

45. The method of claim **34**, wherein said preparing the O-tRNA construct comprises inclusion of one or more tRNA flanking sequences that functionally interact with an RNA polymerase of the cell.

46. The method of claim **34**, wherein the O-tRNA construct comprises an A box eukaryotic transcriptional control element.

47. The method of claim **34**, further comprising mutating a prokaryotic tRNA sequence to include a functional A box eukaryotic transcriptional control element.

48. The method of claim **47**, wherein said mutating comprises site directed mutagenesis.

49. The method of claim **34**, wherein the O-tRNA construct or O-muTrpRS construct comprises: a reporter tag or a purification tag.

50. The method of claim **34**, wherein the O-muTrpRS construct and the O-tRNA construct comprise the same construct.

51. The method of claim **34**, wherein the O-tRNA recognizes a selector codon in a nucleic acid sequence encoding the peptide, thereby incorporating the unnatural amino acid into the peptide.

52. The method of claim **34**, further comprising transfecting a nucleic acid encoding the peptide into the cell.

53. The method of claim **52**, wherein the cell comprises a eukaryotic cell or mammalian cell.

54. The method of claim **34**, further comprising expressing the O-muTrpRS construct or the O-tRNA construct.

55. The method of claim **54**, further comprising purifying expressed O-muTrpRS or expressed O-tRNA.

56. A mammalian cell comprising:

an orthogonal aminoacyl-tRNA synthetase (O-RS) selected from the group consisting of: an orthogonal tryptophanyl-tRNA synthetase (O-TrpRS), an orthogonal mutant tryptophanyl-tRNA synthetase (O-muTrpRS), and a derivative thereof; and,

an orthogonal tRNA (O-tRNA);

wherein the O-RS preferentially aminoacylates the O-tRNA with an amino acid or unnatural amino acid.

57. The mammalian cell of claim **56**, wherein the O-RS comprises a nucleic acid comprising a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 1, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

58. The mammalian cell of claim **56**, wherein the O-RS comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 2, and a conservative substitution thereof.

59. The mammalian cell of claim **56**, wherein the O-tRNA is not substantially aminoacylated by an endogenous aminoacyl-tRNA synthetase of the cell.

60. The mammalian cell of claim **56**, wherein the O-tRNA comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, a conservative variation thereof, and a complementary polynucleotide sequence thereof.

61. The mammalian cell of claim **56**, wherein the unnatural amino acid comprises an amino acid selected from the group consisting of: a tryptophan analog and 5-hydroxy-L-tryptophan (5-HTPP).